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MEMORANDUM

SUBJECT: ***ENDOSULFAN 079401***: Toxicology Chapter for the Reregistration Eligibility Document

CAS No.: 115-29-7

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Attached toxicology chapter for the Reregistration Eligibility Decision (RED) document for endosulfan.

EXECUTIVE SUMMARY

Endosulfan

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 51, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3-benzodioxathiepin-3-oxide) is a chlorinated hydrocarbon. Technical grade endosulfan is a mixture of the alpha and beta isomers. These isomers are in concentration of 70% and 30%, respectively. The database for endosulfan is adequate to assess the toxicology hazard profile and is acceptable to support reregistration. The database includes the required acute toxicity studies; subchronic oral, dermal and inhalation studies; chronic studies, and developmental and reproductive toxicology studies. The mutagenic and carcinogenic potentials of endosulfan were also evaluated. In addition to these studies, metabolism and dermal absorption studies were carried out with radiolabeled endosulfan.

Endosulfan is a chlorinated cyclodiene pesticide, and like other members of this chemical group, the predominant toxicological effect is over stimulation of the central nervous system [by inhibiting Ca^{2+}, Mg^{2+} - ATPase and antagonizing chloride ion transport in GABA (gamma-aminobutyric acid) receptors] with little or no peripheral component. Convulsions (seizures) are the most important symptoms of endosulfan toxicity. Characteristic clinical signs following acute exposure are indicative of central nervous system (CNS) disturbances or over stimulation and include, hyperactivity, uncoordination, seizures, convulsions and death. Although these effects were not generally observed at the LOAEL, at higher doses, they were observed in the acute and subchronic toxicity studies and developmental studies in the rat and rabbit. In a chronic feeding study, dogs also exhibited central nervous system disturbances such as abnormal righting reflexes, tonic contractions, involuntary muscle movements and pronounced sensitivity to noise and light.

Endosulfan is highly acutely toxic via the oral and inhalation routes of exposure, with LD_{50} and LC_{50} values placing it in Toxicity Category I. By the dermal route, however, endosulfan was less toxic (Toxicity Category III). Further, endosulfan is an eye irritant in rabbits (Toxicity Category I) but is not a dermal irritant or sensitizer.

The subchronic toxicity of endosulfan was evaluated in two 13-week feeding studies in the rat and mouse, two 21-day dermal toxicity studies in the rat, and one 21-day inhalation study, also in the rat. In general, females are more sensitive to the toxic effects than males. In the 13-week feeding studies, anemia occurs (consisting of decreased hemoglobin and/or decreased mean red blood cell hemoglobin concentration) at the LOAEL and higher doses in both rats and mice. Treatment-related hematological effects (anemia), however, were not observed in any of the 21-day dermal or inhalation studies. In the dermal studies in rats increased mortality was observed at the LOAEL. In one of the dermal studies, other toxic effects at the LOAEL included increased incidence of liver abnormalities in males and females and increased absolute spleen weight in females. In the other 21-day dermal toxicity study, females had hypersalivation (CNS effect) at the LOAEL. In the 21-day inhalation toxicity study, the LOAEL was established by decreased body-weight gain and decreased leukocyte counts in the males and increased creatinine values in the females.

The chronic toxicity of endosulfan was evaluated in a combined two-year feeding/oncogenicity study in rats, a one-year feeding study in dogs, and an oncogenicity study in mice. Chronic toxicological endpoints at the LOAEL included, in part, decreased body weight gain in male and female rats and decreased body weight in male dogs. Additional effects at the LOAEL included neurological effects in female dogs, marked progressive glomerulonephrosis (kidney toxicity) in male and female rats and blood vessel aneurysms in males rats. Endosulfan did not exhibit any oncogenicity in rats or mice.

The developmental toxicity of endosulfan was evaluated rats and rabbits. Maternal toxicity at the LOAEL included decreased body weights in rats and rabbits and increased incidence of clinical signs in rats (tonoclonic convulsions, increased salivation, mortality) and rabbits (convulsions, rapid breathing, salivation, hyperactivity, mortality). Developmental toxicity in the rat included a slight increase in the incidence of fragmented thoracic vertebral centra and a slight increase in the occurrence of microsomic fetuses. No developmental toxicity was observed in rabbits. There are no indicators of any special sensitivity to the fetus in either the rat or rabbit study; the LOAELs for developmental toxicity were equal to or greater than the LOAELs for systemic maternal toxicity.

The reproductive toxicity of endosulfan was evaluated in a two-generation study in the rat. LOAELs for parental systemic and developmental toxicity were established at the high-dose tested. The LOAEL for parental systemic toxicity was based on decreased body weight and for developmental toxicity, increased pituitary and uterine weights. The increases in pituitary gland weights are suggestive of possible effects on hormonal metabolism and endocrine function. The increased incidence of parathyroid hyperplasia in male rats in the carcinogenicity study and several open literature publications also suggest that endosulfan has hormonal effects.

Endosulfan was evaluated in an acute neurotoxicity screening battery in the rat and an acute delayed neurotoxicity study in the hen. The LOAEL in the rat study was based on behavioral disturbances such as increased incidences of stilted gait, hunched posture, irregular respiration, and decreased spontaneous activity in males and females; females also had increased incidence of straddled hindlimbs, panting and bristled coat. The acute delayed neurotoxicity study in the hen showed no evidence of progressive nerve damage in the brain, spinal cord and peripheral nerve.

Endosulfan was not carcinogenic and did not show any mutagenic potential. There was no increase in the frequency of tumors in either the rat or mouse carcinogenicity studies. Endosulfan is classified as a Group E carcinogen (evidence of non-carcinogenicity for humans) by the Agency. The submitted mutagenicity studies have satisfied the data requirements for mutagenicity testing, and there is no concern for a mutagenic effect in somatic cells. In the *in vitro* or *in vivo* mutagenicity studies, both the mouse lymphoma forward mutation assay and the unscheduled DNA synthesis assay were negative.

Studies with radiolabeled endosulfan evaluated the metabolism in the rat and mouse and dermal absorption in the rat. Endosulfan was found to be rapidly metabolized into mainly water-soluble compounds and eliminated with very little absorption in the gastrointestinal tract.

The primary metabolites include endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan alpha-hydroxy ether, and endosulfan lactone. The metabolites accumulated in tissues, especially in the kidney and liver. Following dietary exposure to endosulfan, a large amount of endosulfan sulfate was recovered in the liver, small intestine and visceral fat with a trace of this metabolite in the muscle. Dermal absorption studies in male and female rats showed that endosulfan is slowly absorbed through the skin and is slowly excreted which suggests that endosulfan bioaccumulates in the body. A dermal absorption factor of 45% was used for assessment of occupational and residential exposure.

The Hazard Identification Assessment Committee (HIARC) of the Health Effects Division identified a subchronic neurotoxicity screening battery in the rat as a data gap. Further, depending on the results of the subchronic neurotoxicity study, the requirement for a developmental neurotoxicity study is being held in reserve. Because of this data gap, an FQPA safety factor of 3X has been retained for protection of infants and children.

HUMAN HEALTH EFFECTS

The toxicology data base for endosulfan is sufficient to support the Reregistration Eligibility Decision (RED).

The following data gaps are indicated at this time.

Series 82-7 (870.6100).	Subchronic neurotoxicity screen
Series 83-6 (870.6300).	Developmental neurotoxicity screen (depending on the outcome of the 870.6100 study)

1. Toxicology Assessment

A. Acute Toxicity

Endosulfan is highly acutely toxic via the oral route, with oral LD_{50} values ranging from 10 to 40 mg/kg in rats. It is practically non-toxic via the dermal route, with a reported dermal LD_{50} value of 2000 mg/kg in rabbits. Endosulfan is very toxic via inhalation, with a reported inhalation LC_{50} range between 0.16-0.5 mg/L for 4 hours exposure. Endosulfan is not a dermal sensitizer and it is an eye irritant and a slight skin irritant. The alpha-isomer is considered to be more toxic than the beta-isomer.

Table 1. Summary of the acute toxicity studies for technical endosulfan.

Guideline#	Study Type	MRID	Results	Tox Category
870.1100	Acute Oral (50% WP)	41183502	LD_{50} = 82 mg/kg in ♂ LD_{50} = 30 mg/kg in ♀	I
870.1200	Acute Dermal (50% WP)	41183503	LD_{50} = 2000 mg/kg	III
870.1300	Acute Inhalation (50% WP)	41183504	LC_{50} = 0.16-0.5 mg/L	II
870.2400	Primary Eye Irritation (50% WP)	41183505	Eye irritant (Residual opacity at day 13)	I
870.2500	Primary Skin Irritation (50% WP)	41183506	Non-irritant	IV
870.2600	Dermal Sensitization	41183507	Not a dermal sensitizer	

Subchronic toxicity (870.3100).

In a 13-week feeding study (MRID#00145668), five groups of Sprague-Dawley 25 rats/sex/dose were fed endosulfan (97.2%) at 0, 10, 30, 60 and 360 ppm (0, 0.5, 1.5, 3.0 and 18 mg/kg/day). Twenty rats/sex/group were sacrificed at week 13 and the remaining 5

rats/sex/group was sacrificed after an additional 4 weeks recovery period. No treatment-related increases in mortality and clinical signs were noted. Body weights were slightly decreased ($p<0.05$ at weeks 6-13 for males and at weeks 1 through 13 for females) in males and females dosed at 18 mg/kg as compared to the controls. The food consumption and food efficiency values were not significantly affected.

There were statistically significant decreases in hemoglobin concentrations, and red blood cell counts in males and females dosed at 3 and 18 mg/kg at almost all intervals (weeks 6, 13 and 17). Statistically significant decreases in erythrocytic indices (mean corpuscular hemoglobin concentration, mean corpuscular volume) verified the hematological changes in males and females dosed at 1.5, 6.0 and 18 mg/kg. Statistically significant decreases of plasma cholinesterase (ChE) activity at week 6 (17%) and week 13 (40%) noted in females dosed at 18.0 mg/kg. The absolute kidney weights were increased at weeks 13 and recovery week 17 in male rats dosed at 3.0 and 18 mg/kg. The absolute liver weights were significantly increased in males dosed at 18 mg/kg at weeks 13 and 17 and in females dosed at 18 mg/kg at week 13. In males dosed at 18 mg/kg, there were statistically significant increases in relative kidney, liver and spleen weights during weeks 13 through recovery week 17. Females dosed at 18 mg/kg had significant increases in relative liver and kidney weights at week 13. Male rats dosed at 1.5, 3.0, and 18 mg/kg also had increased spleen weight.

Microscopically, increased incidences of yellowish discolored and granular/ clumped pigmentation in proximal convoluted tubules of the kidneys were noted to varying degrees in males dosed at 0.5, 1.5, 3.0 and 18 mg/kg at week 13 sacrifice. In female rats, increased traces of discolored and granular/clumped pigmentations in the proximal tubules of the kidneys were noted at 3.0 and 18 mg/kg. Male rats dosed at 1.5, 3.0 and 18 mg/kg had dose related increased incidences of kidney abnormalities of the cortical areas of tubular basophilia (20%, 30% and 65%, respectively) by week 13. The kidney effects persisted in the two highest dosed males through the recovery period.

Increased incidences of urine discoloration (darker) were noted in the 3.0 and 18 mg/kg rats; this occurrence may be related to the increased incidences of pigmentations (yellowish discoloration and granular/clumped pigment) observed in the proximal convoluted tubules in the 18 mg/kg rats. Also in the spleens in females dosed at 18.0 mg/kg there was a minimum to moderate occurrence of hemosiderosis (fragmental red blood cell deposits) that may indicate probable red blood cell destruction.

Based on the results of this study, the kidney and liver are the target organs of toxicity. Also red blood cell (RBC) destruction occurred possibly as a result of the damage to the kidneys.

This was evidenced by red blood cell indices (hemoglobin and RBC counts) decreases. Spleen weights were increased along with the occurrence of hemosiderosis which may have resulted by the removal of the damaged RBCs. The LOAEL was 1.5 mg/kg/day, based on kidney abnormalities and increased spleen weight in males rats; the NOAEL was 0.5 mg/kg/day.

The study was classified as acceptable and satisfied the guideline requirements for a subchronic feeding study in rats (870.3100).

In another 13-week feeding study (MRID#00147182), five groups of 20 CD-1 mice/sex/dose were fed dietary endosulfan (97.2%) at 0, 2, 6, 18 and 54 ppm (0, 0.24, 0.74, 2.13 and 7.30 mg/kg/day in males and 0, 0.27, 0.80, 2.39 and 7.52 mg/kg/day in females).

Increased mortality occurred in male (60%) and female 50%) mice of the highest dose group between week 2 and week 12. The majority of deaths occurred between weeks 4 and 9 for males and weeks 2 and 7 for females. The report noted that one male and one female had convulsions and salivation prior to death during week 5. Most likely the deaths were due to the proconvulsant properties of the chemical. There were no treatment related adverse effects of endosulfan on the body weight, hematological parameters or clinical chemistries. Male mice of the highest dose group had increased relative heart and liver weights and increased absolute spleen weight. Female mice had increased absolute liver weight compared to control. There were no histopathological findings to support the increases in organ weights. At necropsy, surviving female mice dosed at the highest dose tested had higher incidences of vascular congestion (30%) and hemorrhaging of the lung (15%). The LOAEL was 7.3 mg/kg/day, based on high incidences of mortality in both males and females; the NOAEL in males was 2.1 mg/kg/day.

This study was classified as acceptable, and satisfied the guideline requirements for 90-day feeding study in mice (870.3100).

Subchronic dermal toxicity studies

In a dermal toxicity study (MRID#41048506), three groups of 11 male and 11 female Wistar rats were dermally treated with formulated endosulfan (49.5% purity) for 30 days for a total of 21 applications of 0, 160, or 640 mg/kg/day (males) or 0, 80, or 160 mg/kg/ day (females). Another group of 6 rats/sex were treated with 40 mg/kg for the same duration. Following the dosing period, groups of five rats/sex/dose (control, mid-, and high-dose groups) were kept without treatment for a 25-day recovery period. The other 6 rats/sex/dose were

sacrificed at the end of the 30 days.

Three females from the 160 mg/kg dose group died days 3, 11, and 21, respectively. One female given 80 mg/kg died on day 21 of study. There were statistically significant decreases in body weights and body weight gains in high-dose males from day 10 to study termination; body weights decrements continued during the recovery period. Except early in the study, body weights and body weight gains of the treated females were not affected. There were no treatment related changes in organ weights or histopathology. Slight dermal erythema, dryness, and scales were noted in mid- and high-dose males and females beginning on days 6 and reversed by the end of the recovery period. There were no macroscopic or microscopic dermal changes.

Reticulocyte counts of mid- and high-dose males and high-dose females were increased. There was no corrolating decrease in red blood cell (RBC) counts, hemoglobin concentration or hematocrit to account for the increase in reticulocyte counts. Levels of cholesterol and total lipids in high-dose females were increased following dosing.

Serum cholinesterase activity of mid- and high-dose females was statistically significantly depressed (28% and 46%, respectively) on day 30 of study and remained depressed at the end of the recovery period. The serum cholinesterase activity of mid and high-dose males were slightly depressed (both are 13% of controls) on day 30 of study but were not statistically significantly. The RBC or brain cholinesterase activity values were not affected in either male or female rats. The mechanism of action for the anticholinesterase property of this organochlorine is not the same as that of organophosphates or carbamates, that is, irreversible phosphorylation/ carbamoylation of acetylcholinesterase causing an accumulation of acetylcholine, overstimulation of cholinergic receptors, and consequent clinical signs of cholinergic toxicity. Although the exact mechanism by which endosulfan produces cholinesterase inhibition has not been elucidated, it is a weak cholinesterase inhibitor as evidenced by the high doses needed to inhibit the enzyme.

The NOAEL was 160 mg/kg in males and 40 mg/kg in females. The systemic LOAEL was 640 mg/kg in males, based on body weight loss and 80 mg/kg in females, based on mortality and decreased serum ChE activity.

This study is acceptable and satisfied the guideline requirements for a subchronic dermal toxicity study in rats (870.3200).

In a dermal toxicity study (ACC#257682 and 257683), endosulfan (97.2%) was applied dermally to five groups of 11 male and 11 female Wistar rats at doses of 0, 12, 48, 96, and 192 mg/kg in males and 0, 3, 6, 12, and 48 mg/kg in females for 21 applications over 30 days. Six rats/sex/group were kept as the main treatment group for 29 days, and then sacrificed. After

treatment for 29 days, the remaining five rats/sex/group were kept another 14 days before they were sacrificed.

Two males dosed at 192 mg/kg (one each on days 6 and 9) and four females dosed at 48 mg/kg (between days 2 and 22) died following tonoclonic convulsions. One female from each of the groups dosed at 3, 6, and 12 mg/kg died on day 18 from unknown causes. Treatment-related signs of toxicity (piloerection, salivation and lacrimation) were noted in 96 mg/kg males. One female given 12 mg/kg had piloerection, slight lacrimation and autoaggression (biting at the bandage). Eight females dosed at 48 mg/kg showed treatment-related clinical signs (three showed hypersalivation, 2 of which died; one had crusted eyes; one had bloody crusted nose which subsequently died; one had dacryohemorrhea, tonic convulsions, marked salivation and bloody exudate). Skin dryness and desquamation were noted in all dosed groups. Scattered erythema and edema were also noted, but they disappeared at the end of the treatment period. The body weight, food consumption, hematology, and urinalysis values were not affected by the treatment. There were significant differences in a number of clinical chemistry parameters, including cholinesterase inhibition, compared to the controls, however, these changes were not considered toxicologically significant because they were sporadic and/or dose-related trends were not evident. As seen in another dermal study in rats (MRID 41048506) the highest dose in male rats (192 mg/kg) depressed serum ChE activity (33%) compared to the controls. Again the high dose that inhibited serum cholinesterase in male rats produced convulsions and death.

On day 30, there were statistically significant increased relative kidney weights in the males dosed at 12, 96, and 192 mg/kg; the absolute kidney weights, however, were not statistically significantly increased. Discrete deposition of pigments in a few cells of the proximal straight tubule of the kidneys was noted; this finding is of interest, because similar pigment deposition in the proximal convoluted tubules was noted in other subchronic studies with endosulfan*.

*Special studies (MRID# 40767601) were conducted by the registrant to determine the source of the kidney pigmentation and found conclusively that pigments were due to metabolites of endosulfan (endosulfan sulfate and endosulfan lactone) that were temporarily stored in kidneys. Further support came from special staining of the kidneys with Prussian Blue to failed to detect the presence of ferritin (evidence of hemosiderosis).

In male rats, the NOAEL was 96 mg/kg/day and the LOAEL was 192 mg/kg/day, based on increased mortality and serum ChE activity inhibition. In female rats, the systemic NOAEL was 12 mg/kg/day and the LOAEL was 48 mg/kg/day, based on mortality and increased incidence neurotoxic clinical signs.

This study was classified as acceptable, and satisfied the guideline requirements of a

repeated dermal toxicity study in rats (870.3200).

In a 21-Day dermal toxicity study (ACC#: 257684, 257685) , endosulfan (97.2% w/w) was applied dermally to five groups of six male and six female Wistar rats at doses of 0, 1, 3, 9, and 27 mg/kg/day and six males only at 81 mg/kg/day, for 21 applications (5 days a week) over 30 days. Five of the six high-dose females died on days 2 and 6 of the study. Three of the six high-dose males died on days 2 and 3 of study (females were not tested at this dose). Two of the three 81 mg/kg/day males that died had tonoclonic convulsions, increased salivation and respiration. Although no deaths occurred in males dosed at 27 mg/kg/day, 2 of the 6 males dosed at 9 mg/kg/day died on days 5 and 8. Prior to death, one male rat had central nervous system signs that included, salivation, blood-encrusted nose, dyspnea and staggered gait. There were no treatment related changes in clinical chemistry and hematology parameters. Changes in liver cells started at 9 mg/kg/day dose levels and above. Liver abnormalities included enlargement of parenchymal cells in peripheral sections, together with a loss of cytoplasmic basophilia, isolated cell necroses, and frequent mitoses. Females dosed at 9 mg/kg/day had significantly increased absolute and relative spleen and absolute adrenal weights, compared to controls. Slight dermal irritation occurred in all groups and at all evaluation intervals. It appears that dermal irritation was more persistent in females at 3 and 9 mg/kg/day dose groups, as evidenced by greater dermal irritation scores (2-3 times) than that of controls.

For systemic toxicity, the NOAEL was 3 mg/kg/day and the LOAEL was 9 mg/kg/day based on increased mortality in males, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes.

This study was classified as acceptable, and satisfied the guideline requirements of a repeated dermal toxicity study in rats (870.3200).

21-day inhalation study- rats.

In a range-finding study (MRID 41667501) two groups of 5 Wistar rats/sex were exposed, nose-only, to aerosol concentrations of endosulfan (97.2%) at 0.0024 and 0.0065 mg a.i./L for 6 hours/day, five days/week for a total of 7 exposures. Two females exposed to 0.0065 mg/L died by Day 8 of the study. Female survivors had clinical signs including, tremors, trembling, tonic-clonic convulsions and reduced corneal reflexes. Males exposed to the highest concentration were ataxic and had irregular breathing. Body weight loss were noted in males and females at both concentrations early in the study (days 3-4). Based on the results of this range finding study, the highest concentration for the subchronic study was set at 0.0020 mg a.i./L. In

a 21-day inhalation toxicity study (MRID# 00147183), ten male and ten female Wistar rats were exposed, nose-only, to technical endosulfan (97.2% pure) at concentrations of 0 (air), 0.0005, 0.0010, and 0.0020 mg/L air (0.097, 0.194, and 0.387 mg/kg/d)[1] for 6 hours/day, 5 days/week for a total of 21 exposures over 29 days. An additional group of 5 animals/sex/dose were held for a 4-week recovery period after receiving the test aerosol. No mortality or clinical signs of toxicity occurred during the study. Group mean body weights were similar to controls with the exception of males in the highest dosed group that had lower body weight (3-5%) from day 20 through 29. In the highest dosed males from the recovery group, the decrements in body weights were more pronounced (12-16%) from recovery days 34-60. Although neither sex had any statistically significantly body weight changes during the exposure period and the number of recovery animals for each sex was only 5, the apparent effect suggested a possible delay in its manifestation.

Erythrocyte counts in the low and mid dose males at the end of the exposure period (Day 29) were significantly elevated. No effects on erythrocyte counts were observed at the high dose, hence the changes did not demonstrate a pattern of toxicity because the toxicological significance of an increased RBC count is unknown. In addition, the test report stated that the values were apparently within the norm for the species and strain studied. Some slight effects on clinical chemistry and in hematology counts were noted but these did not demonstrate significant toxicity of the test compound. There were statistically significant decreases in leucocyte counts (20.1%) in the high-dose males, which seemed to be marginally dose related but did not indicate significant toxicity. High-dose females had increased creatinine (21%) values suggestive of kidney toxicity and were judged to be treatment related but there were no other supporting kidney toxicity in histopathology or organ weight changes.

The NOAEL was 0.0010 mg a.i./L (0.20 mg/kg/day), the LOAEL was 0.0020 mg a.i./L (0.40 mg/kg/day), based on decreased body-weight gain and decreased leukocyte counts in the males and increased creatinine values in the females.

The subchronic (21-day) inhalation study (MRID# 00147183) was upgraded to acceptable, based on the additional submitted data/information noted. This study satisfied the guideline requirement (870.3455) for a subchronic inhalation toxicity study in rats.

C. Chronic toxicity.

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[1] Conversion of mg/L to oral dose (mg/kg/day) = mg/L X absorption (1.0) X[Respiratory Volume (Wistar rats) for 6 hours/day] X Duration of Exposure (5days/wk)/ body weight X 7 days/week

$$= \frac{0.001 \text{ mg/L} \times 1.0 \times [8.46(\text{RV}) \times 6 \text{ hrs}) \times 5 \text{ d/wk}}{0.187 \text{ kg} \times 7 \text{ d/wk}} = 0.194 \text{ mg/kg/day}$$

Chronic Oral Toxicity Study in Dogs

In a chronic feeding study in dogs (MRID# 41099501), five groups of 6 Beagle (BEAK) dogs/sex/group were fed technical endosulfan (96.5% purity; 64% alpha and 32% beta-endosulfan) at 0, 3, 10, 30 and 30/45/60 ppm (0, 0.65, 1.75, 0.65-1.30 in males and 0, 0.57, 1.75, 0.65-1.30 in females) for one year. The highest dose group was given 30 ppm initially, increased to 45 ppm after 54 days and adjusted to 60 ppm after 106 days.

All dogs in the high-dose (30/45/60 ppm) groups had pronounced nervous symptoms after they were dosed at 60 ppm dose level; extreme sensitivity to noise and optical stimuli and tonic contractions of the muscles of the extremities, face, and chaps were observed. No spontaneous deaths occurred. One high-dose male that showed poor general health was sacrificed on day 126 and another high-dose male was sacrificed on day 146 because of poor general health and with edematous swelling of the knee joints. All other high-dose dogs were sacrificed on days 146 and 147, because of increasing neurological effects. The body weights of the 3 and 10 ppm dose groups were comparable with the controls. The body weight gain of the 30 ppm males was decreased starting from week 46 on, and the overall body weight to week 54 was 28% and 1% lower than the controls, for the males and females, respectively. Body weights of the high-dose dogs started to decrease after the dose was increased to 60 ppm at week 15. The mean body weight gain reductions during the 0-21 weeks were 48% and 28% for the high-dose males and females, respectively. Only the food consumption values of the 30 ppm and high dose groups were affected due to the unpalatable taste of the feed. One 30 ppm male refused to eat prior to sacrifice and one high-dose male decreased its food intake from weeks 16 to 21. Overall food consumption of the high-dose dogs was reduced by 10% as compared to the controls.

Neurological effects were only noted in the high-dose dogs. Two males showed a deficit of righting and placing reaction after stimuli; one was sacrificed on day 126 and the other one on day 145. Four of the six high-dose dogs had similar symptoms prior to terminal sacrifice.

No treatment-related hematological effects were noted, except that the hematocrit values of the high-dose males and females were slightly increased at weeks 6 and 12. No treatment-related changes in clinical chemistry, urinalysis, organ weights, and gross macroscopic changes were noted. No dose-related depression of plasma and RBC ChE activities were noted. Because of the wide range of variations in the brain ChE activity values that were noted in most groups, no conclusions can be made. Histological findings were generally unremarkable and incidental, except for one dog (30 ppm) with gross and microscopic lung changes, and one high-dose treated dog with a pulmonary edema; both were sacrificed before termination.

Based on the results of this study, the systemic NOAEL is 10 ppm (0.65 and 0.57 mg/kg/day in males and females, respectively). The systemic LOAEL 30 ppm (≈ 1.75 mg/kg/day) based on decreased body weight gain in males and increased incidences of neurologic findings in males and females (loss or weakening of placing and righting reactions, tonic contractions of abdominal muscle and masticatory muscles a few hours after feeding).

This study was classified as acceptable and satisfied the guideline requirements for a chronic feeding study in dogs (870.4100).

D. Carcinogenicity.

Chronic/Carcinogenic Feeding Study in Rats

In a combined chronic/oncogenicity study (MRID# 41099502), groups of 50 Sprague-Dawley rats/sex/group were fed dietary technical endosulfan (97.1% purity) at 0, 3.0, 7.5, 15.0, and 75.0 ppm (0, 0.1, 0.3, 0.6, and 2.9 mg/kg/day for males and 0, 0.1, 0.4, 0.7, and 3.8 mg/kg/day for females) for 104 weeks. A satellite group of twenty rats/sex were dosed in a similar fashion and were used for hematology and clinical chemistry evaluations. No treatment-related clinical signs, mortality, food consumption and urinalysis were observed. Mean body weights of the males and females dosed at 75.0 ppm were statistically significantly decreased ($p<0.01$; 17.6%) as compared to their respective controls. Grossly, enlarged kidneys were noted in females in the satellite group dosed at 75.0 ppm (8/20 *versus* 2/20 in the controls).

No treatment-related changes were noted in the clinical chemistry and hematology parameters evaluated. Marginal decreases of leukocyte (at week 26) and lymphocyte counts (at weeks 26 and 52) were noted in the males dosed at 75.0 ppm. At week 13, RBC counts and MCV values were decreased in all treated females as compared to the controls. Since dose related trends were not evident and since no changes were noted at other intervals, these changes were not judged to be related to treatment. Increased incidences of blood vessel aneurysms (18/70 *versus* 10/70 in controls) and enlarged lumbar lymph nodes (19/70 *versus* 14/70 in controls) were noted in the male rats dosed at 75.0 ppm as compared to the controls. Increased incidences of enlarged kidneys were seen in females dosed at 75 ppm (30/70 *versus* 21/70 in controls) as compared to the controls. Other organ weights were not affected by dosing. Although slightly decreased testes weights were observed in males dosed at 15 and 75 ppm, these changes were not considered toxicologically significant.

Histopathologically, increased incidences of blood vessel aneurysms (18/70 *versus* 9/70 in controls) were noted in male rats dosed at 75.0 ppm. Also, a statistically significant increased

incidence of marked progressive glomerulonephrosis occurred in the kidneys of male (30/70 *versus* 20/70 in controls) and in female (8/70 *versus* 1/70 in controls) rats dosed at 75.0 ppm. The incidence of the glomerulonephrosis in the kidneys in the high-dose males (43%) was higher than that observed in the historical controls data (reported at 19.7%). This data was re-evaluated because of some concerns expressed by one member of the RfD/RfC Work Group (Memorandum: L Taylor to G. Ghali, March 19, 1993). It was stated in this memo that the increase in the severity of progressive glomerulonephrosis in rats of both sexes at the high-dose level was regarded as an adverse effect and that the spontaneously occurring renal disease was exacerbated by exposure to the test material. No treatment-related neoplastic lesions were evident in this study. A slight increased incidences of pituitary adenoma in males and females dosed at 75 ppm and fibroma/ adenoma of the mammary glands females dosed at 75 ppm were not judged to be related to treatment, because dose-related trends were not evident The doses used in this study appear to be adequate to test the carcinogenic potential of the test compound, as evidence by the compound-related systemic effects noted above.

Based on the results of this study, the systemic NOAEL is 15.0 ppm (0.6 and 0.7 mg/kg/day for males and females, respectively) and the systemic LOAEL is 75.0 ppm (2.9 and 3.8 mg/kg /day for males and females, respectively) based on decreased body weight gain in males and females, enlarged kidneys in females and increased incidences of marked progressive glomerulonephrosis in male and female rats and blood vessel aneurysms in males.

This study was classified as acceptable, and it satisfied the guideline requirements for a combined chronic/oncogenicity study in rats (870.4300).

Chronic/Carcinogenic Feeding Study in Mice

In a carcinogenicity study (MRID# 40792401), four groups of HOE:NMRKf (SPF71) 60 mice/sex/group were fed technical endosulfan (97.9% purity) at 0, 2, 6 and 18 ppm (0, 0.3, 0.9 and 2.6 mg/kg/day) for 24 months. A satellite group of twenty mice/sex/group was dosed in a similar fashion and was used for hematology and clinical chemistry evaluations.

No treatment-related effects were evident in clinical signs, food consumption, hematology, clinical chemistry, urinalysis, organ weights and gross and microscopic evaluations. At study termination, statistically significant increased mortality was noted in the high-dose females (72% mortality *versus* 55% of the controls). Mean body weights of the males and females were comparable among all groups. At 12 months, the lung and ovary weights of the 18 ppm females were significantly ($p<0.05$) decreased and at 18 months, the relative liver weights of males dosed at 18 ppm and relative ovary weights in females dosed at 18 ppm were slightly but significantly

decreased. At 24 months, organ weights were comparable among all groups. Decreases in organ weights noted at various intervals during the study are not judged to be related to treatment, because they are within the normal historical ranges. Histopathologically, slight increased incidences of epithelial thickening of the urinary bladder were noted in all treated males (0, 5, 8 and 12, in the control, low-, mid- and high-dose groups, respectively) and females (0, 6, 9 and 10, in the control, low-, mid- and high-dose groups, respectively). The original reviewer of this study concluded these increases were not toxicologically significant, because of the "absence of a progression to a clear proliferative change". Lymphosarcoma was found practically in all organs of male and female mice and since it was noted equally among all dose groups, these occurrences are judged to be spontaneous and strain-related changes, and were not considered to be treatment-related effects. The systemic NOAEL is determined to be 6 ppm (0.9 mg/kg/day), and the systemic LOAEL is 18 ppm (2.65 mg/kg/day), based on increased incidences of mortality in females.

This study was classified as acceptable/ guideline and it satisfied the guideline requirements for an oncogenicity study in mice (870.4200). It is not acceptable for a combined chronic/oncogenicity study in mice because some clinical chemistry parameters were not evaluated.

E. Developmental toxicity.

Developmental Study in Rats

In a developmental toxicity study (MRID#43129101), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female Wistar rats at doses of 0 (sesame oil), 0.7, 2.0, and 6.0 mg/kg/day from days 7 through 16 of gestation. Four pregnant dams (one after 6 treatments, one after 8 treatments and two after 10 treatments) and two non-pregnant dams (on test days 14 and 17) at the highest dose died. Tonoclonic convulsions were observed in three of the four dams that died. Of the surviving dams at this highest dose, 13 had tonoclonal convulsions and 3 had increased salivation which lasted from 1 to 3 days. Body weight was slightly but statistically significantly decreased in the high dose dams during days 14 to 17 of gestation. In addition, food consumption was statistically significantly decreased during the first week and second week of dosing in the high dose dams.

There was a slight increase (not statistically significant) in the number of fetuses/litter weighing less than 3 grams in the high dose group. There was a statistical significant increase in

the number of high dose fetuses with fragmented thoracic vertebrae centra compared to control (6.3% vs 0.7%. This was considered a minor anomaly. There were no treatment related effects in the number of corpora lutea/dam, implantations/dam, live fetuses/dam, resorptions/dam dead fetuses/dam, pre-or post-implantation losses, litter weight, fetal body weight, or fetal crown-rump length among the groups. No other significant fetal malformations were noted. The maternal toxicity LOAEL was 6.0 mg/kg/day, based on 80 % mortality, tonoclonic convulsions, increased salivation, and decreased bodyweight gains and food consumption; the NOAEL was 2.0 mg/kg/day. The developmental toxicity NOAEL is also set at 2.0 mg/kg/day and the developmental toxicity LOAEL is 6.0 mg/kg/day, based on a slight increase in the incidence of fragmented thoracic vertebral centra and a slight increase in the occurrence of fetuses/litter weighing less than 3 grams. There are no indicators of any special sensitivity to the fetus in this study.

This study was a repeat study for an unacceptable developmental toxicity study (ACC# 243707). This study was classified as acceptable (guideline), and satisfied the guideline requirements for a developmental toxicity study in rats (870.3700).

Developmental Study in Rabbits

In a developmental toxicity study (MRID#: 00094837), endosulfan technical (97.3% a.i.) was administered by gavage to four groups of 20 pregnant female New Zealand White rabbits at dose levels of 0, 0.3, 0.7, and 1.8 mg/kg/day from days 6 through 28 of gestation. The does were sacrificed on gestation day 29. Four does dosed at 1.8 mg/kg/day died on gestation days 7, 10, 21 and 29. Only one of the three does that died was considered a treatment related effect. Three of them were due to improper oral gavage as evidenced by the presence of oil in the trachea and the lungs and one doe dosed at 1.8 mg/kg/day that died showed evidence of hemorrhagic activity. Increased incidences of convulsions, rapid breathing, salivation and hyperactivity were also noted in does dosed at 1.8 mg/kg/day. Body weight losses were noted in does dosed at 0.7 and 1.8 mg/kg/day during days 19-29 but these values were not statistically significant. The body weight (after corrected for uterine weight) was only negative in does dosed at 1.8 mg/kg/day compared to the controls. No treatment-related effects on fetal deaths/resorptions, altered growth, developmental anomalies and malformation were noted. Developmental toxicity was not observed at any dose level.

The maternal LOAEL was 1.8 mg/kg/day, based on a decreased bodyweight, as well as increased incidences of deaths, convulsions, rapid breathing, salivation and hyperactivity; the maternal NOAEL was 0.7 mg/kg/day. The developmental NOAEL was the highest dose tested (1.8 mg/kg/day) There were no indicators of any special sensitivity to the fetus in this study.

This study was classified as acceptable-guideline and it satisfied the guideline requirements for a developmental toxicity study in rabbits (870.3700).

F. Reproductive toxicity in Rats (multi-generation).

In a 2-generation reproduction study (MRID#: 00148264), four groups of Sprague Dawley rats (32 rats/sex) were fed dietary endosulfan (97% purity) during premating, through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm (0, 0.2, 1.0, and 5.0 mg/kg/day in males and 0, 0.2, 1.2, and 6.2 mg/kg/day in females) for two generations. Parental (F_0) and F_{1b} generations were mated twice. Mortality, food/water consumption, and body weight were not affected in either generation, but there was a decrease in body-weight gain in the parental (F_0) females at the high-dose level during the first week of study (67% of control). Pregnancy rate, gestation times, the ability to rear young to weaning, and pre-coital time were comparable among the groups at both matings in both generations. Parental (F_0) males had increased heart weight at the mid- and high-dose levels and increased liver and kidney weights at the high-dose level. Parental females had increased brain and liver weights at the high-dose level. In the F_{lb} adults, the high-dose males had increased kidney weights compared to the controls and the females had increased liver weights at the mid- and high-dose levels. These organ weight changes were not considered to be toxicologically significant (see notes below regarding RfD Committee memo dated October 13, 1992). There were no apparent adverse effects of treatment on litter size or litter/pup weight at birth at either matings. The litter size of both matings of both generations was not affected by any dose. In the first mating of the F_0 generation, there was an increase in the cumulative litter loss (8 litters) at the high-dose level. Litter and pup weights were comparable at birth among all dose groups in both generations, but there was a decrease in litter weight during the lactation to weaning period in both matings in the F_0 generation, which was significant at the high-dose level in the first mating and at the mid- and high-dose levels in the second mating (dose-related). Because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, the decrease in litter weight was not considered to be treatment-related. Increased pituitary weights (high-dose female pups of F_O generation) and increased uterine weights (high-dose female pups of F_{lb} generation) were observed in the offspring. There were no histopathological findings observed that could be attributed to treatment. Although there were no significant effects noted on the dams, the dose levels were considered adequate, based on the results of the range-finding study in which there was an increase in cumulative pup loss and a reduction in litter size at the 100 ppm dose level at days 24 and 28 days post weaning.

The NOAEL for parental toxicity was 15 ppm (1.2 mg/kg/day), and the parental LOAEL was 75 ppm (6.2 mg/kg/day), based on decreased body weight. The NOAEL for reproductive effects was 75 ppm (6.2 mg/kg/day), the highest dose tested. The reproductive LOAEL is

greater than 75 ppm (6.2 mg/kg/day). The developmental toxicity NOAEL was 15 ppm (1.2 mg/kg/day), and the developmental toxicity LOAEL was 75 ppm (6.2 mg/kg/day), based on increased pituitary and uterine weights. The effects at the high dose level cannot be considered as an indicator of any special sensitivity to the pup, because the biological relevance of these effects is unclear.

This study was classified as acceptable-guideline, and satisfied the guideline requirements (870.3800) for a 2-generation reproduction study in rats.

G. Neurotoxicity Testing.

Acute neurotoxicity screen in rats

In a neurotoxicity study (MRID#44403101), male and female Wistar rats (10/sex/dose) were fasted overnight and then orally gavaged once with endosulfan (98.6%) suspended in 2% starch mucilage at a constant volume of 10 ml/kg body weights. Two separate control groups of 10 rats/sex were used in the study. One control group was assigned to males, dosed at 25, 50 and 100 mg/kg and females dosed at 3, 6 and 12 mg/kg. The other control group was assigned to males dosed at 6.25 and 12.5 mg/kg and females at 0.75 and 1.5 mg/kg. Rats were observed for 15 days and survivors were sacrificed at week three. The treated groups were dosed at levels of 0 (vehicle), 6.25, 12.5, 25, 50 and 100 mg/kg for the males and 0 (vehicle), 0.75, 1.5, 6 and 12 mg/kg for the females. The animals were evaluated for neurobehavioral effects (FOB and motor activity) on day 7 prior to dosing, and days 1 (within 8 hours after dosing), 8 and 15 of post-dosing. Neuropathological examinations were carried out at terminal sacrifice (at week 3) on ten rats/sex of controls and four 100 mg/kg male rats and five 12 mg/kg female rats.

Six males dosed at 100 mg/kg and one female dosed at 12 mg/kg died were found dead on the day of dosing. Treatment-related clinical signs were noted from 4 to 8 hours after dosing on Day 1 (peak-time of effects) in males at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. These symptoms disappeared after day 2 in all survivors. Clinical signs noted included tonoclonic convulsions, decreased spontaneous activities, stilted gait, stupor, prone position, squatting posture, straddled hindlimbs, bristle coat, eyelid narrowing, irregular respiration and panting in males dosed at 50 and 100 mg/kg and females dosed at 6 and 12 mg/kg. In males given 25 mg/kg and females given 3 mg/kg there was increased incidences of stilted gait, squatting posture, irregular respiration and decreased spontaneous activities. Animals with “drawn in flanks” were only noted in females dosed at 3, 6, 12 mg/kg. Tremors were noted in three and four females dosed at 6 mg/kg and 12 mg/kg, respectively and in four males dosed at 50 mg/kg. Salivation was noted in one male dosed at 100 mg/kg, and in one female each dosed at 6 and 12 mg/kg. The

observed clinical effects are consistent with the mechanism of endosulfan neurotoxicity (inhibition of brain gamma-amino-butyric acid (GABA) receptors) of CNS activation and convulsions. No compound-related effects on motor activity were noted for rats that survived. No treatment-related effects were seen on: the rearing frequency, fore-and hind-limb grip strength, and on landing foot-spread; body weight and food consumption; organ weight; gross pathology; or histo(neuro) pathology. The NOAEL was 12.5 mg/kg for males and 1.5 mg/kg for females. The LOAEL was 25 mg/kg for males based increased incidences of stilted gait, squatting posture, and irregular respiration, as well as decreased spontaneous activity. The LOAEL was 3 mg/kg for females, based on an increased incidence of stilted gait, squatting posture, straddled hindlimbs, irregular respirations, panting and bristled coat and decreased spontaneous activity.

This study was classified as acceptable (guideline) and it satisfied the Subdivision F guideline requirements for a neurotoxicological screening study for rats (870.6200).

Additional information from the literature (These references were not submitted to the Agency by the Registrant)

1. Lakshmana and Raju (1994). Endosulfan induces small but significant changes in the levels of noradrenalin, dopamine and serotonin in the developing rat brain and deficits in the operant learning performance. *Toxicology*, Vol. 91(2):139-50.

Endosulfan was administrated via gastric intubation to Wistar rat pups of both sexes at 6 mg/kg body weight/day from post-natal days 2-25. Its effect on levels of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) was assayed in olfactory bulb (OB), hippocampus (HI), visual cortex (VC), brainstem (BS) and cerebellum (CB) on days 10 and 25 using high-performance liquid chromatography (HPLC). The activity of acetylcholinesterase (AChE) was also estimated in the same regions of the brain. Performance in operant conditioning for solid food reward was assessed in 25-day-old rats. NA levels were increased in OB (12%) and BS (10%) at 10 days of age and in HI (20%) and CB (12%) at 25 days of age. DA levels were decreased in HI at both 10 (42%) and 25 (45%) days. Serotonin levels were increased in OB (12%), HI (41%), VC (30%) and BS (15%) at 10 days of age but at 25 days, levels were decreased in BS (20%) and CB (31%). The activity of AChE was not different from the control groups in any of the regions studied. The investigators suggested that monoaminergic systems in the developing rat brain respond to endosulfan by undergoing something like a 'reorganization'. However, such changes do not ameliorate certain functional losses following the exposure to endosulfan as operant conditioning revealed deficits in acquisition as well as retention of

memory.

2. Gilbert, M.E., 1992. A characterization of chemical kindling with the pesticide endosulfan. Neurotoxicology and *Teratology*, Mar; 14(2):151-158.

Repeated administration (3 times per week for a total of 21 doses) of endosulfan (5 and 10 mg/kg in corn oil, po) was found to induce behavioral seizures in rats. Behavioral seizure development was most apparent in the high dose group (10 mg/kg). Heightened seizure responsiveness to a challenge dose was maintained following a two-week, drug-free period, arguing against cumulative toxicity as a mechanism for seizure induction. An enhancement in the rate of kindling was also evident in the low dose group (5 mg/kg) in the absence of clonic seizure development during dosing. Chemical kindling with endosulfan may result from the interaction of this pesticide with GABA-mediated neurotransmission in the central nervous system (CNS).

3. Gilbert, M.E., 1992. Proconvulsant Activity of Endosulfan in Amygdala Kindling. Govt Reports Announcements & Index, Issue 17; *Jnl. of Neurotoxicology and Teratology*, v14: 143-149.

The proconvulsant properties of the chlorinated hydrocarbon insecticide, endosulfan, were investigated using electrical kindling of the amygdala. Male rats were implanted with electrodes in the amygdala and stimulated once daily with a standard kindling stimulus 60-90 minutes following endosulfan (0, 2.5, 5.0 mg/kg, PO). No alterations were observed in either the threshold to induce an after discharge (AD) or the duration of clonus upon seizure generalization. The results suggest that faster kindling rates induced by endosulfan are not readily attributable to transient toxicant-related increases in excitability of the nervous system. It was concluded that endosulfan has proconvulsant properties that may be related to an action on GABA within the central nervous system.

4. Blanco-Coronado, J.L., Repetto, M., Ginestal, R.J., Vicente, J.R., Yelamos, F., and Lardelli, A. 1992. Acute intoxication by endosulfan. J. Clin. Tox., 30(4): 575-583.

The authors report six patients with acute endosulfan intoxication. The symptoms were nausea, vomiting, headache, and dizziness beginning from ½ to 2.7 hours after ingestion. Acute intoxication involved: a) gastrointestinal symptoms, tonic-clonic convulsions, respiratory depression, metabolic acidosis and hyperglycemia and hemodynamic instability appears within 4 hours of ingestion, b) pulmonary edema and pulmonary aspiration, consumption coagulopathy and decreased platelets, elevated serum transaminases and persistent hemodynamic instability can develop.

H. Mutagenicity (Series 84-2).

The HIARC considered that the submitted mutagenicity studies have satisfied the data requirements at this time and there is no concern for a mutagenic effect in somatic cells. The following studies, all classified as acceptable represent the mutagenicity data base for endosulfan.
Chromosome aberrations

Gene Mutation in Mammalian Cells in Vitro.: Under the conditions of a mutagenicity evaluation of endosulfan in the mouse lymphoma forward mutation assay (MRID#: 00148266), six doses of endosulfan (97.2%) ranging from 6.25 to 50 µg/ml without S9 activation and seven doses from 6.25 to 100 µg/ml with S9 activation induced a significant increase in mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma. Although an increase was detected at a single dose of 100 µg/ml in the S9 activation system, the response was considered to be spurious because of a low cloning efficiency associated with a very high toxicity (8.6% relative growth). The positive control 3-methyl-cholanthrene (MCA) at doses of 2.5 and 4 µg/ml clearly demonstrated the sensitivity of the S9-activated system to detect a mutagenic response at doses comparable to those of the test compound. The positive control ethylmethanesulfonate (EMS) at 0.25 and 0.4 µg/ml, although used at doses well above those of the test article, demonstrated the capability of the assay to detect a mutagenic response. The test compound, therefore, is considered to be non-mutagenic in the mouse lymphoma forward mutation assay. This mouse lymphoma mutation assay is acceptable. This study satisfied one of three mutagenicity study categories (870.5300).

Unscheduled DNA synthesis: The cytotoxicity and UDS assay were performed in parallel (MRID#00148265). Fifteen test concentrations ranging from 1020 to 0.102 µg/ml were used. Endosulfan (97.2%) was soluble up to 51 µg/ml and was toxic at this concentration level. Survival decreased from 102.8% at 0.255 µg/ml to 31.5% at 25.5 µg/ml. The positive control (0.10 µg/ml 2-AAF) was weakly toxic (85.7%) and induced an unscheduled DNA synthesis. Under conditions of the assay, doses of technical endosulfan (97.2% purity), ranging from 25.5 to 0.102 µg/ml, did not induce an appreciable change in the pattern of nuclear labeling of rat hepatocyte. These doses resulted in a cell survival range of 31.5 to 105.5%. Endosulfan is considered inactive in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay. This primary rat hepatocyte unscheduled DNA synthesis (UDS) assay is classified as acceptable. This study satisfied one of the mutagenicity study categories
(870.5550).

I. Metabolism

General Metabolism

Endosulfan is rapidly degraded into mainly water-soluble compounds and eliminated in mammals with very little absorption in the gastrointestinal tract. In rabbits, the beta-isomer is cleared from blood plasma more quickly than the alpha-isomer, with reported blood half-lives of approximately 6 hours and 10 days, respectively, which may account in part for the observed differences in toxicity. The metabolites are dependent on the mixture of isomers and the route of exposure. Most of the endosulfan seems to leave the body within a few days to a few weeks.

Two biotransformation pathways have been described for endosulfan in animals. The first pathway involves hydrolysis and oxidation to various sulfur-free metabolites, namely endosulfan diol, endosulfan ether, hydroxyendosulfan ether, endosulfan lactone and polar conjugates. The second pathway involves the formation of various sulfur-containing metabolites, namely endosulfan sulfate, endosulfan sulfuric acid ester and endosulfan dicarbonic acid. Studies in the rat suggest that those components excreted primarily in the feces after single administration of a single dose of endosulfan, are unchanged parent compound, endosulfan sulfate and all of the sulfur-free metabolites. The urine contained mainly endosulfan diol and polar conjugates in addition to some unchanged parent compound.

In a study in rats (MRID#050037030) endosulfan metabolites accumulated in tissues, especially in the kidney and liver. In rats, metabolites of endosulfan include endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan alpha-hydroxy ether, and endosulfan lactone. In another study (MRID#00004257) when mice were fed endosulfan, a large amount of endosulfan sulfate was recovered in the liver, small intestine and visceral fat with a trace of this metabolite in the muscle.

Dermal absorption/penetration

In a dermal absorption study (MRID#40223601), three groups of 24 male Crl: CD(SD) Br rats/group were treated topically with radiolabeled endosulfan suspension (94.6% purity) at nominal doses of 0.1, 1.0, and 10 mg/kg and exposed for 0.5, 1, 2, 4, 10 and 24 hours. The application site was shaved and then cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin, 5 hours before dosing. The compound was applied onto the application site (3.7 cm in diameter = 10.8 cm^2). After exposure, the application site was washed with 5 ml of mild soap solution and three 5 ml portions of water for further analysis. The animals were sacrificed and the application sites were washed with 5 ml of 1% liquid ivory soap and three 5 ml portions of water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain and fat were analyzed for the presence of radio labeled compound. The percent doses absorbed over a 24-hour period were 2.2-21.6, 0.32-21.52, and 0.08-8.38 for the 0.1, 1.0, and 10 mg/kg dose groups, respectively. The percentages of endosulfan

absorbed at 1, 10 and 24 hours intervals, were 1.8, 7.6 and 21.6% for rats dosed at 0.1 mg/kg, 0.57, 5.77 and 21.52%, for rats dosed at 1.0 mg/kg, and 0.29, 3.86, and 8.38% for rats dosed at 10 mg/kg. The percent doses remaining in/on the skin after soap and water washes over a 24-hour period were 62.1-56.5, 78.1-57.7, and 80.2-66.7 for the 0.1, 1, and 10 mg/kg dose groups, respectively. This data showed that significant portions of the dose remained on the skin of male rats following soap and water wash was performed. At 24-hour interval, the data showed that endosulfan bioaccumulated in the body of the rats.

This study was classified as acceptable and satisfied the data requirements for a dermal absorption study in rats (870.7600).

b. In another dermal absorption study (MRID#41048504), three groups of 16 female Crl:CD(SD)BR rats/group were applied topically with radiolabeled endosulfan (purity 94.6%) at nominal doses of 0.1, 1, and 10 mg/kg (1.9, 21.9, and 231.4 mg/cm^2) to determine the fate of the residue that was left in/on the skin following 10 hours of exposure. The application sites were shaved one day before dosing. Thirty minutes before dosing the sites were cleaned with acetone to remove surface fats and oils and to extract some lipoid from the skin. A rubber ring was glued on the shaved application site, then the compound was applied onto an application site within the rubber ring, and afterwards a filter paper was cemented on the rubber ring. Ten hours after dosing, the application sites were washed with 1% liquid Ivory soap and rinsed with water. The skin wash, filter paper, rubber ring, application site and adjacent skin, untreated skin, liver, kidney, brain, fat, muscle, blood, urine, feces, and carcass were analyzed for the presence of radio labeled compound. The radioactive labeled endosulfan presence was analyzed in four live rats/group at 24, 48, 72 and 168 hours after dosing. The percent doses absorbed at 24 hours were 22.1, 16.1 and 3.8% and at 168 hours were 44.8, 46.4 and 20.3% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The percentages of the doses remaining on/in the skins at 168 hours were 41.4, 56.2 and 72.8% for the 0.1, 1, and 10 mg/kg dose groups, respectively. The data showed that endosulfan bioaccumulated in the body of the rats.

The HIARC selected the dermal absorption factors of 45 % (rounded of 44.8%) at 168 hours post exposure.

J. Other Issues

Reports in the open literature suggest that endosulfan may affect hormone metabolism and endocrine function. In studies submitted to the Agency, treatment-related effects were seen in the two-generation reproduction study in rats (MRID#00148264) characterized as increases in the pituitary glands weights and uterine weights in female offspring that may affect the neuroendocrine

control. In a chronic feeding study in rats, high doses of endosulfan induced testicular atrophy with necrosis of the germinal lining of the seminiferous tubules which could result in possible spermatogenesis. In a carcinogenicity study, there was increased incidences of parathyroid hyperplasia in male rats.(MRID#41099502). Male rats fed high doses of endosufan for 15 and 30 days had significantly inhibited testicular androgen biosynthesis. In *in vitro* bioassays, endosulfan was shown to have estrogenic properties comparable to those of DDT and chlordecone and binds to the progesterone receptor as well as estrogen receptor. Though this was not heavily weighted since the Agency is still in the process of developing guidance concerning endocrine disruption, it was also taken into consideration. A summary review on the potential endocrine-related effects are appended to this Toxicology Chapter.

Toxicity Endpoint Selection

On September 1, 1998 the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of endosulfan, established acute and chronic Reference Doses (RfDs), evaluated the carcinogenic and mutagenic potential and selected the toxicological endpoints for occupational as well as residential exposure risk assessments. The HIARC also addressed the potential sensitivity of infants and children from exposure to Endosulfan as required by the Food Quality Protection Act (FQPA) of 1996.

On January 11, 2000, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-evaluated the toxicology database for Endosulfan to select toxicity endpoints for short, intermediate and long-term occupational/residential dermal and inhalation exposure risk assessments. The toxicology endpoints selected for acute and chronic dietary risk assessments remained the same. The Committee's conclusions are summarized in the following table.

Table III. Summary of doses and toxicological endpoints selected for various exposure scenarios.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL=1.5	Increased incidences of convulsions seen within 8 hours after dosing in females at 3.0 mg/kg	Acute neurotoxicity-Rat
	UF=100	Acute RfD = 0.015 mg/kg	
Chronic Dietary	NOAEL = 0.6	Reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats at 2.9 mg/kg.	2-year chronic toxicity/ carcinogenicity-Rat
	UF=100	Chronic RfD = 0.006 mg/kg/day	

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	
Short-Term (Dermal)	Dermal NOAEL= 3.0	Hepatotoxicity (enlargement of parenchymal cells ,loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg	21-day dermal toxicity-Rat	Target MOE = 100
Intermediate-Term (Dermal) [a] **Up to 30 Days**	Dermal NOAEL= 3.0	Hepatotoxicity (enlargement of parenchymal cells ,loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg	21-day dermal toxicity-Rat	Target MOE = 100
Long-Term (Dermal) [a] **Longer than 30 Days**	Dermal NOAEL= 3.0	Hepatotoxicity (enlargement of parenchymal cells ,loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes at 9 mg/kg	21-day dermal toxicity-Rat	Target MOE = 300
Short-Term (Inhalation)	Inhalation NOAEL= 0.001 mg/L (0.20 mg/kg/d)[2]	Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.002 mg/L (0.40 mg/kg/d)	21-day inhalation-Rat	Target MOE = 100
Intermediate-Term (Inhalation) **Up to 30 Days**	Inhalation NOAEL= 0.001 mg/L (0.20 mg/kg/d)	Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.002 mg/L (0.40 mg/kg/d)	21-day inhalation-Rat	Target MOE = 100
Long-Term (Inhalation) **Longer than 30 Days**	Inhalation NOAEL= 0.001 mg/L (0.20 mg/kg/d)	Decreased body-weight gain and decreased leukocyte counts in males and increased creatinine values in females at 0.002 mg/L (0.40 mg/kg/d)	21-day inhalation-Rat	Target MOE = 300

a =Since an Oral NOAEL was selected, a dermal absorption factor of 45% should be used for route-to-route extrapolation

b =Since and Oral NOAEL was selected, an inhalation absorption (100%) factor should be used for route-to-route extrapolation.

G. FQPA Considerations

Based on hazard assessment, the HIARC recommended to the FQPA Safety Committee, that 10X factor for the protection of infants and children should be reduced to 3X because:

1) developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits;

2) the two generation reproduction toxicity study in rats showed no increased susceptibility in pups when compared to adults; and

3) there was no evidence of abnormalities in the development of fetal nervous system in the pre/post natal studies. Neither brain weight nor histopathology (perfused or nonperfused) of the

[2] Conversion of mg/L to oral dose (mg/kg/day) = mg/L X absorption (1.0) X[Respiratory Volume (Wistar rats) for 6 hours/day] X Duration of Exposure (5days/wk)/ body weight X 7 days/week

$$= \frac{0.001 \text{ mg/L} \times 1.0 \times [8.46(\text{RV}) \times 6 \text{ hrs}) \times 5 \text{ d/wk}}{0.187 \text{ kg} \times 7 \text{ d/wk}} = 0.194 \text{ mg/kg/day}$$

nervous system was affected in the subchronic and chronic toxicity studies.

However, there is a datagap for a subchronic neurotoxicity study in rats. Data from this study will be used (in conjunction with other studies) in determining the need for a developmental neurotoxicity study (which is currently placed in reserve status). The developmental neurotoxicity study will provide additional data (e.g., functional parameter development, potential increased susceptibility, effects on the fetal nervous system etc.).

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Appendix A:
ENDOSULFAN
Evidences of Endocrine-related Effects
(David Liem 11/24/98)

The EDSTAC (1996) defines an "endocrine disruptor" as an exogenous substance that changes endocrine function and causes adverse effects at the level of the organism, its progeny, and/or (sub)populations of organisms.

Below is a summary of endocrine-related effects as it relates to endosulfan. Evidence of endocrine-related effects are based on data of submitted guideline studies and from the literature.

Conclusions

Based on the evidence and effects on the endocrine glands as well as the results of hormonal changes presented below, it could be concluded that endosulfan may affect endocrine systems as some other organochlorine pesticides which have estrogenic and enzyme-inducing properties.

In a standard chronic feeding study, testicular atrophy was noted in male rats dosed at 20.4 mg/kg/day, characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis (MRID#00004256). It was not clear whether the degeneration and necrosis of the germinal cells lining of the seminiferous tubules of the male rats were relate to endocrine related effects. Decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6- phosphate dehydrogenase) were seen in male rats dosed with endosulfan at 2.5 mg/kg/day through oral intubation for 70 days (Sinha, et al, 1995). In another rat study, oral administration of endosulfan at 2.5 mg/kg for 60 days resulted in a slight decreased testes weight (Ansari et al, 1984).

Increased pituitary weight in female pups in the Fo generation and increased uterine weights in female pups of the F1b generation were noted in a 2-generation rat study at 6.18 mg/kg/day dose level (MRID#00148264). In addition, parathyroid hyperplasia was found in male rats dosed at 20.8 mg/kg/day in a chronic feeding study (MRID#00004256). In a special acute rat study, intraperitoneally injection of endosulfan at 4.1 mg/kg resulted in thyroid follicle damage (Cerkezkayabekir et al, 1997).

Adverse effects on the ovaries and adrenal glands were not reported in experimental animals.

It should be noted that the Acute RfD for endosulfan was based on a NOAEL of 1.5 mg/kg, based on increased incidences of convulsions in female rats at 3 mg/kg in an Acute Neurotoxicity Study in Rats (MRID No.:44403101). The Chronic RfD was based on a NOAEL of 0.6 mg/kg/day, based on reduced body weight gain and increased incidences of marked progressive glomerulonephrosis and blood vessel aneurysms in male rats dosed at 2.9 mg/kg/day in a Combined Chronic/Carcinogenicity Study in Rats (MRID#41099502).

Evidences of endosulfan affecting the hormonal system based on changes of estrogenic, androgenic, and progesteronic activities are as follows:

1. Evidence of Estrogenic Effects

In an *in vitro* bioassay (E-screen test) using human breast estrogen-sensitive MCF7 cells, endosulfan was shown to have estrogenic properties comparable to those of DDT and chlordecone, which are known to be estrogenic in rodent models. Thus, endosulfan has estrogenic effects on human estrogen-sensitive cells (Soto, et al, 1994). For endosulfan the Relative Proliferative Effect was reported between 77 - 81% that of estradiol (Soto, et al., 1995).

The recombinant yeast bioassay was used for the screening and determining the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. The competitive binding assays were performed to determine the direct interaction between rtER (estrogen receptor) and xenobiotics. In this study, endosulfan exhibited estrogenic activity in the two bioassays (Petit et al, 1977).

In a competition binding assays with a synthetic progestin [3H]R5020, endosulfan inhibited the binding capacity of [3H]R5020 to aPR (progesterone receptor). These results provided evidence supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses (Vonier et al, 1996).

Although there are questions regarding the validity or reliability of the *in vitro* testing methods, it should be noted that the results of these assays on known "*endocrine disruptor"* chemicals revealed consistent results among the three assays used (the receptor binding, the transcriptional activation, and the *in vivo* effect in an estrogen-responsive tissue) in respect to what is known about the estrogenic activities of the chemicals tested and their requirements for metabolic activation (Shelby et al, 1996).

2. Evidence of Androgenic Effects

Male adult rats fed endosulfan (po) at 7.5 and 10 mg/kg body weight dose levels for 15 and 30 days, significantly inhibited testicular androgen biosynthesis. No appreciable alterations were apparent in body weights, testicular wet weights, and cytosolic and microsomal protein contents of testes in treated rats (Singh et al, 1990).

Endosulfan lactone caused the greatest reduction in binding of androgen to the androgen receptor, alpha endosulfan and endosulfan sulfate caused slight reduction in binding at high concentrations (1 mM) when tested in a cell-free in vitro binding assay using cytosolic prostate tissue extracted from mature rats and ^{3}H-methyltrienolone, a synthetic androgen (Brieske, 1997).

Chronic endosulfan exposure in rats led to a considerable increase in the activities of drug metabolizing enzymes, whereas it had inhibitory effect on the activities of enzymes involved in the androgen biotransformation (Singh, 1989).

3. Evidence of Progesteronic Effects

The mammalian sperm acrosome reaction (AR) is essential to fertilization. The acrosome reaction (AR) can be initiated *in vitro* by progesterone, a putative physiological initiator that helps to activate sperm GABA receptor/chloride channels, and by glycine. Glycine is a substitute for the egg zona pellucida, which activates sperm

glycine receptor/chloride channels. At 1 nM (0.41 ng/ml or 0.41 ppb), chlordane and endosulfan, chlorinated cyclodiene blockers of insect neuronal GABA receptor/chloride channels, strongly inhibited the AR (acrosome reaction) initiated by progesterone or glycine. Inhibitory concentrations of these cyclodienes are well within the range detected in human and wildlife tissue and fluids as a result of environmental contamination (Turner, 1997).

The ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of alligators was evaluated. A competition binding assay with the synthetic progestin (3H)R5020 was conducted to assess the ability of chemicals to interact with aPR. This assay showed that endosulfan inhibited (3H)R5020 binding to aPR (progesterone receptor). There is evidence that environmental chemicals bind with the aER and aPR of the American alligator, supporting the hypothesis that the reported reproductive abnormalities in American alligators may be related to modulation of endocrine-related responses (Vonier, 1996).

Table 2. Summary Table of the Effects of Endosulfan on Endocrine Glands

Organ/Hormone Affected	Study/Species	Dose mg/kg/d	Notes
Testes Weight↓	* Rat - 60-day study	2.5	* Slightly increased
	* Rat - 70-day study	2.5	* Accompanied by decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6-phosphatedehydrogenase)
	* Rat - Chronic study	20.8	* Testicular atrophy characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multi-nucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis.
Uterine Weight↑	*Rat - Two-generation study	6.18	*Female pups of the F1b generation
Pituitary Gland Weight ↑	*Rat - Two-generation study	6.18	* Female pups in the Fo generation
Parathyroid Hyperplasia ↑	* Rat - Chronic study	20.8	* In males
Thyroid follicle damage ↑	* Rat - Acute intraperitoneal Injection	4.16	* Follicle damage

I. Evidence Affecting Endocrine Glands and Functions

A. Testes:

1. Two groups of 50 Osborne-Mendel rats/sex/group, were given endosulfan (98.8%) in the diet at the time weighted average concentration of 408 and 952 ppm to males (20.4 mg/kg/day and 40.8 mg/kg/day) and 223 and 445 ppm (11.1 mg/kg/day and 22.0 mg/kg/day) to females, respectively. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks.

Dose-related depression in the rates of growth and survival were shown in the male rats. At week 54, 52% of the high-dose males died (the Tarone test for a positive dose-related trend in mortality was highly significant). The low- and high-dose male rats were terminated during week 74 and week 82, respectively. No appreciable difference in mean body weight among the females was noted. At termination (week 102), 70% of the controls, 62% of the low-dose and 50% of the high-dose groups survived.

At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes. In the males, 47/50 and 43/47 were observed in the low- and high-dose (20.4 and 40.8 mg/kg/day), respectively. In the females, 27/50 and 29/50 toxic nephropathy was also observed low- and high-dose (11.1 and 22.0 mg/kg/day), respectively.

A parathyroid hyperplasia was reported to be associated with renal lesions and occurred in 21/48 low-dose (20.4 mg/kg/day) and in 18/47 high-dose (40.8 mg/kg/day) males. Only 1/49 parathyroid lesion was noted in the low dose female.

Testicular atrophy was noted in 3/19 controls, 18/47 low-dose (20.4 mg/kg/day) , and 24/47 high-dose (40.8 mg/kg/day) male rats; this testicular atrophy was characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis.

In the high dose male rats early mortality, associated with toxic nephropathy, was noted. Probably as a result of a high mortality rate, the incidence of tumors in the males was higher in the controls as compared to the low- or high-dose groups. Early deaths of the male rats preclude the usefulness of any analysis of late developing tumors.

This study was classified as unacceptable guideline study for a carcinogenicity study in rats (NCI, 1978. Bioassay of Endosulfan for Possible Carcinogenicity. (CAS# 115-29-7) and NCI-CG-TR-62. Report. Hazelton Lab. Inc., Vienna, Virginia. DHEW Publ# (NIH) 78-1312. NCI Technical Report No.62, 1978. MRID#:00004256).

2. Adult male rats were exposed to 0, 2.5, 5.0 or 10.0 mg endosulfan/kg body weight through oral intubation for 70 days. Decreased sperm counts in the cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes (sorbitol dehydrogenase, lactic dehydrogenase, gamma glutamyl transpeptidase, and glucose-6-phosphatedehydrogenase) were seen in all the endosulfan-dosed groups. Endosulfan caused impairment in testicular functions by altering activities of the enzymes responsible for spermatogenesis, thereby influencing intratesticular spermatid count and causing low sperm production and sperm deformity (Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan-induced biochemical

changes in the testis of rats. Vet Hum Toxicol 1995 Dec; 37(6):547-549).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

3. Endosulfan was administered orally (2.5 and 7.5 mg/kg) daily to male rats for a period of 60 days. The distribution pattern of alpha- and beta-isomers was studied using a gas-liquid chromatograph equipped with an electron capture detector. There was a significant increase in liver and lung weights. The testes weight was slightly decreased. At both dose levels, the concentration of alpha-isomer was highest in kidney (574 and 1655 ng/g, respectively), followed by lung, ventral prostate, spleen, testes and brain. In the seminal vesicle, epididymis, heart and liver, the concentration of beta-isomer was higher than the alpha-isomer. The results of the study indicated a differential ability to accumulate the two isomers of endosulfan which may help to explain the difference in the toxic potential of the alpha- and beta-isomers [Ansari R.A.; Siddiqui M.K.J.; Gupta P.K. Toxicol. Lett., (1984) 21/1 (29-33)].

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

B. Ovaries

No evidence of any treatment-related effects was found in animals studies.

C. Pituitary Glands:

1. In a 2-generation reproduction study (MRID#: 00148264), exposure of Crl:COBS CD(SD)BR rats to Endosulfan (97% purity) via the diet during premating and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm (0, 0.20, 1.00, and 4.99 mg/kg/day in males and 0, 0.24, 1.23, and 6.18 mg/kg/day in females), produced minimal maternal toxicity at the high-dose level. There were 32 rats/sex/group in the F_O generation and 26 rats/sex/group in the F1 generation.

Increased pituitary weights (high-dose female pups of 1st mating in F_O generation) and increased uterine weights (high-dose female pups of 1st mating of F_{1b} generation) were observed in the offspring. There were no histopathological findings observed that could be attributed to treatment.

The NOEL for parental toxicity is 15 ppm (1.23 mg/kg/day), and the parental LOEL is 75 ppm (≈ 6.18 mg/kg/day), based on decreased body weight.The NOEL for reproductive effects is 75 ppm (6.18 mg/kg/day), the highest dose tested. The reproductive LOEL is greater than 75 ppm (6.18 mg/kg/day).The developmental toxicity NOEL is 15 ppm (1.23 mg/kg/day), and the developmental toxicity LOEL is 75 ppm (6.18 mg/kg/day), based on increased pituitary and uterine weights.

This study was classified as acceptable-guideline, and it satisfied the guideline requirements (83-4) for a 2-generation reproduction study in rats. (Edwards, J.A., et al, 1984. Effect of Endosulfan-Technical (Code 02671 0 I AT209) on Reproductive Function in the Rat. Hoechst Aktien-gesellschaft. Huntingdon Research Centre, Study#: HST204/83768. July 19, 1984. HED Doc# 004881, 008868, 009552; Tox. Chem.#: 420; ACC#: 256127; 41799301; TRID#: 460002-031; MRID#: 00148264).

D. Thyroid/Parathyroid Glands:

1. Two groups of 50 Osborne-Mendel rats/sex/group, were given endosulfan (98.8%) in the diet at the time weighted average concentration of 408 and 952 ppm to males and 223 and 445 ppm to females, respectively. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks.

Dose-related depression in the rates of growth and survival were shown in the male rats. At week 54, 52% of the high-dose males died (the Tarone test for a positive dose-related trend in mortality was highly significant). The low- and high-dose male rats were terminated during week 74 and week 82, respectively. No appreciable difference in mean body weight among the females was noted. At termination (week 102), 70% of the controls, 62% of the low-dose and 50% of the high-dose groups survived. At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes. In the males, 47/50 and 43/47 were observed in the low- and high-dose, respectively. In the females, 27/50 and 29/50 toxic nephropathy was also observed low- and high-dose, respectively. A parathyroid hyperplasia was reported to be associated with renal lesions and occurred in 21/48 low-dose and in 18/47 high-dose males. Only 1/49 parathyroid lesion was noted in the low dose female.

Testicular atrophy was noted in 3/19 controls, 18/47 low-dose, and 24/47 high-dose male rats; testicular atrophy is characterized by degeneration and necrosis of the germinal cells lining of the seminiferous tubules, multinucleated cells (fusions bodies), and calcium deposition resulting in aspermatogenesis. In the high dose male rats early mortality, associated with toxic nephropathy, was noted. Probably as a result of a high mortality rate, the incidence of tumors in the males was higher in the controls as compared to the low- or high-dose groups. Early deaths of the male rats preclude the usefulness of any analysis of late developing tumors.

This study was classified as unacceptable guideline study for a carcinogenicity study in rats. (NCI, 1978. Bioassay of Endosulfan for Possible Carcinogenicity. (CAS# 115-29-7) and NCI-CG-TR-62. DHEW Publ# (NIH) 78-1312. NCI Tech. Rept No.62, 1978. MRID#: 00004256).

2. In a non-guideline study, the acute toxic effects of endosulfan on thyroid glands were investigated by light and electron microscopy. Endosulfan (LD-30 4.16 mg/kg) was injected intraperitoneally into adult male mice. In the light microscopic studies, it was observed that some of the follicles had been damaged and joined together. Furthermore, colloidal fluids had diffused from follicles and dispersed into connective tissues. Swelling in endoplasmic reticulum sacs, expansion in perinuclear area, pycnotic nuclei and accumulation of heterochromatin were determined by electron microscopy (Cerkezkayabekir A; Aktac T. Turkish Journal of Biology 21 (4). 1997. 439-444.

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

E. Adrenal Glands

No evidence of any treatment-related effects were found in animals studies.

II. Evidence Affecting Hormonal Activities

There are several hormones that affect sexual and reproductive functions of an animal include estrogen, progesterone, and androgen including testosterone.

A. Evidence Affecting Estrogen Activities:

Estrogens are hormones produced by humans and other animals and are secreted primarily by the ovaries (female). Estrogens are defined by their ability to induce the proliferation of cells of the female genital tract. Estrogens stimulate sexual maturation of females, and play an important role in reproductive function. Hormones act through specific receptors. A complex feedback mechanism provides balance. Mammals have both "male" and "female" hormones.

Studies that provide evidence that endosulfan affecting the normal estrogen functions are:

1. The E-SCREEN assay was developed to assess the estrogenicity of environmental chemicals using the proliferative effect of estrogens on their target cells as an end point. This quantitative assay compares the cell number achieved by similar inocula of MCF-7 cells in the absence of estrogens (negative control) and in the presence of 17 beta-estradiol (positive control) and a range of concentrations of chemicals suspected to be estrogenic. Concentration describes the dose at which an estrogenic effect is detected; maximal cell yield is obtained at concentrations between 10 and 100 pM estradiol. Most xenobiotics are active at 10M. The RPE (Relative Proliferative Effect) measures the ratio between the maximal cell yield achieved by the xenobiotic and that of estradiol. The RPP (Relative Proliferative Potency) is the ratio between the minimal concentration of estradiol and the minimal dose of the xenoestrogen test compound needed to produce maximal cell yields x 100. For endosulfan the concentration detected was at 0.0001 and maximum cell yield obtained at 10 M concentration and the Relative Proliferative Effect was between 77.17-81.25% that of estradiol (Soto, A.M, Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. , and Serrano, F.O. Environ Health Perspect 1995 Oct; 103 Suppl 7:113-122).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

2. An *in vitro* bioassay was used to assess the estrogenicity of several pesticides. The E-screen test uses human breast estrogen-sensitive MCF7 cells and compares the cell yield achieved after 6 days of culture in medium supplemented with 5% charcoal-dextran stripped human serum in the presence (positive control) or absence (negative control) of estradiol and with diverse concentrations of xenobiotics suspected of being estrogenic. Among the organochlorine pesticides tested, toxaphene, dieldrin, and endosulfan had estrogenic properties comparable to those of DDT and chlordecone; the latter are known to be estrogenic in rodent models. The E-screen test also revealed that estrogenic chemicals may act cumulatively; when mixed together they induce estrogenic responses at concentrations lower than those required when each compound is administered alone. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells (Soto, A.M., Chung, K.L., and Sonnenschein, C., 1994. Environ-Health-Perspect; VOL 102, ISS 4, 1994, P380-3).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

3. Reports of reproductive abnormalities in the American alligator from Lake Apopka, Florida, have been

linked to a spill of DDT and other pesticides suspected of having hormonelike activity. To determine whether environmental chemicals had the potential to function as exogenous hormones in the American alligator, the ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of the alligator. In competition binding assays with [3H]17beta-estradiol, some DDT metabolites showed inhibition of [3H]17beta-estradiol binding to aER. A combination of DDTs demonstrated an additive decrease in [3H]17beta-estradiol binding to aER. Modern-use chemicals such as alachlor, trans-nonachlor, endosulfan, and atrazine also competed with [3H]17beta-estradiol for binding to the aER. To test the effect of chemicals identified in alligator eggs from Lake Apopka on [3H]17beta-estradiol binding, we mixed these chemicals at concentrations measured in eggs in the competition binding assay. P,p'-DDD and trans-nonachlor, both found in Lake Apopka, interacted with aER, whereas others such as chlordane and toxaphene did not. Surprisingly, combinations of these chemicals decreased [3H]17beta-estradiol binding in a greater than an additive manner. To assess the ability of chemicals to interact with aPR, we performed competition binding assays with the synthetic progestin [3H]R5020. Most of the chemicals tested did not reduce [3H]R5020 binding to aPR, whereas endosulfan, alachlor, and Kepone inhibited binding. These results provide the first evidence that environmental chemicals bind the aER and aPR from the American alligator, supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses. The findings that combinations of chemicals demonstrated a greater than additive interaction with the aER and some chemicals bind to the aPR in the competition binding assay are novel. This suggests that interactions of these chemicals with the endocrine system are complex (Vonier PM; Crain DA; Mclachlan JA; Guillette LJ Jr; Arnold SF Environ. Health Perspect, (1996). Vol. 104, No. 12, pp. 1318-1322.).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

4. A yeast system highly and stably expressing a rainbow trout estrogen receptor (rtER) is used to analyze the biological activity of the receptor. The recombinant yeast system appears to be a reliable, rapid and sensitive bioassay for the screening and determination of the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals commonly found in the environment. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. Moreover, competitive binding assays were performed to determine direct interaction between rtER and xenobiotics. In this study, 50% of the 49 chemical compounds tested exhibited estrogenic activity in the two bioassays: the herbicide diclofop-methyl; the fungicides biphenyl, dodemorph, and triadimefon; the insecticides lindane, methyl parathion, chlordecone, dieldrin, and endosulfan; polychlorinated biphenyl mixtures; the plasticizers or detergents alkylphenols and phthalates; and phytoestrogens. To investigate further biphenyl estrogenic activity, Its principal metabolites were also tested in both bioassays. Among these estrogenic compounds, 70% were able to activate rtER in yeast and hepatocytes with variable induction levels according to the system. Nevertheless, 30% of these estrogenic compounds exhibited estrogenic activity in only one of the bioassays, suggesting the implication of metabolites or different pathways in the activation of gene transcription. This paper shows that it is important to combine in vivo bioassays with *in vitro* approaches to elucidate the mechanism of xenoestrogen actions (Petit F; Le Goff P; Cravedi J-P; Valotaire Y; Pakdel F, 1977).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

5. Reliable methods for detecting and characterizing estrogenic chemicals are needed. A general agreement should be reached on which tests to use and that these tests should then be applied to the testing of both man-made and naturally occurring chemicals. As a step toward developing a comprehensive approach to screening chemicals for estrogenic activity, three assays for detecting estrogenicity were conducted on 10 chemicals with known or suspected estrogenic activity. The assays were:

1) competitive binding with the mouse uterine estrogen receptor,
2) transcriptional activation in HeLa transfected with plasmids containing an estrogen receptor and a response element, and
3) the uterotropic assay in mice.

The chemicals studied were 7-beta-estradiol, diethylstilbestrol, tamoxifen, 4-hydroxytamoxifen, methoxychlor, the methoxychlor metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), endosulfan , nonylphenol, o,p'-DDT, and kepone. These studies were conducted to assess the utility of this three-assay combination in the routine screening of chemicals, or combinations of chemicals, for estrogenic activity. Results were consistent among the three assays with respect to what is known about the estrogenic activities of the chemicals tested and their requirements for metabolic activation. By providing information on three levels of hormonal activity (receptor binding, transcriptional activation, and an in vivo effect in an estrogen- responsive tissue), an informative profile of estrogenic activity is obtained with a reasonable investment of resources (Shelby M D; Newbold R R; Tully D B; Chae K; Davis V L. Environmental Health Perspectives 104 (12). 1996. 1296-1300).

B. Evidence Affecting the Androgen Activities:

The testes, ovary and adrenal cortex are responsible for the normal synthesis of androgens. In males, this hormone regulates the spermatogenesis and the maturation of sperm in the testes. Testoterone is the dominant steroid testicular hormone. Studies on endosulfan showing evidence on affecting the androgen function are as follows:

1. Endosulfan significantly inhibited testicular androgen biosythesis in adult rats, when fed (po) at 7.5 and 10 mg/kg body weight dose levels, consecutively for 15 and 30 days. No appreciable alterations were apparent in body weights, testicular wet weights, and cytosolic and microsomal protein contents of testes in treated rats. Profound decrease in the levels of plasma gonadotrophins (FSH and LH) along with plasma testosterone and testicular testosterone were observed at both the doses of endosulfan, particularly after the longer exposure of 30 days. Activities of steroidogenic enzymes studied (3beta- and 17beta-hydroxysteroid dehydrogenases) were considerably lowered on longer exposure of endosulfan. A significant decrease in the contents/activities of microsomal cytochrome P-450 and related mixed function oxidases (MFOs) in testes of treated rats was also observed, along with a marked inhibition in the activity of cytosolic conjugation enzyme, glutathione-S-transferase both doses studied. These biochemical changes were reversed when the endosulfan treatment was withdrawn (Singh S K; Pandey R S Indian J Exp Biol, (1990). Vol. 28, No. 10, pp. 953-956).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP cannot be verified.

2. Effects of endosulfan and chlorpyrifos were examined on the growth of neonatal male and female rats reproductive organs and serum testosterone and estradiol concentrations. The rats received subcutaneous sub-lethal

injections daily after seven days of birth for 15 days with one of the following 4.5 mg/kg and 9.0 mg/kg weak and strong doses of endosulfan respectively and 7.0 mg/kg and 14.0 mg/kg weak and strong doses of chlorpyrifos or 1 ml corl oil. Endosulfan and chlorpyrifos did not affect the body weights or mortality. Endosulfan and chlorpyrifos decreased the weights of the male and female reproductive organs and suppressed the testosterone and estradiol concentrations. The insecticides probably suppress the Leydig cell activity of testis and interstitial cell activity of an ovary (Ahmad M M; Ahmad M M; Sarvat S. Pakistan Journal of Zoology, (1993). Vol. 25, No. 1, pp. 11-14).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

3. The potential of endosulfan and its microbial transformation products to cause reproductive disturbances through androgen-receptor mediated activity was determined in a cell-free in vitro binding assay using cytosolic prostate tissue extracted from mature rats and 3H-methyltrienolone, a synthetic androgen. Among the compounds tested, endosulfan lactone caused the greatest reduction in binding of androgen to the androgen-receptor, endosulfan alpha and endosulfan sulfate caused slight reduction in binding at high concentrations (1 mM). Preliminary experimental testing binary combinations of these compounds suggest additive effects (Brieske, J. A.; Mousa, M.; Madhukar, B. V.; Boyd, S. A.; Chou, K. Organohalogen Compound (1997), 34(Dioxin '97), 357-359).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

4. Chronic endosulfan exposure in rats led to a considerable increase in the activities of drug metabolizing enzymes, whereas it had inhibitory effect on the activities of enzymes involved in the androgen biotransformation. Endosulfan also produced a dose- and duration-dependent increase in microsomal lipid peroxidation. The alterations produced after shorter duration showed much variation with respect to the dose levels and exposure period of endosulfan studied. The above biochemical changes were reversed after endosulfan withdrawal (Singh S K; Pandey R S . Indian J Biochem Biophys, (1989). Vol. 26, No. 4, pp. 262-267).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

C. Evidence Affecting the Progesterone Activities:

1. The mammalian sperm acrosome reaction (AR) is essential to fertilization. It can be initiated *in vitro* by progesterone, a putative physiological initiator that helps to activate sperm GABA receptor/chloride channels and by glycine, a substitute for the egg zona pellucida, which activates sperm glycine receptor/chloride channels. Even at 1 nM (0.41 ng/ml or 0.41 ppb), chlordane and endosulfan, chlorinated cyclodiene blockers of insect neuronal GABA, receptor/ chloride channels, strongly inhibited the AR initiated by progesterone or glycine. Inhibition of the latter was also seen at 0.1 nM chlordane and endosulfan, but neither cyclodiene inhibited either AR initiator at 0.01 nM. Inhibitory concentrations of these cyclodienes are well within the range detected in human and wildlife tissue and fluids as a result of environmental contamination (Turner, K.O. Sylvanen, M; Meizel S, 1997. Journal of Andrology, (1997). Vol. 18, No. 6, pp. 571-575).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not

this study was conducted under GLP can not be verified.

2. Reports of reproductive abnormalities in the American alligator from Lake Apopka, Florida, have been linked to a spill of DDT and other pesticides suspected of having hormonelike activity. To determine whether environmental chemicals had the potential to function as exogenous hormones in the American alligator, the ability of chemicals to bind the estrogen receptor (aER) and progesterone receptor (aPR) in a protein extract prepared from the oviduct of the alligator were evaluated. In competition binding assays with (3H)17-beta-estradiol, some DDT metabolites showed inhibition of (3H)17-beta-estradiol binding to aER. A combination of DDTs demonstrated an additive decrease in (3H)17-beta-estradiol binding to aER. Modern-use chemicals such as alachlor, trans-nonachlor, endosulfan , and atrazine also competed with (3H)17-beta-estradiol for binding to the aER. To test the effect of chemicals identified in alligator eggs from Lake Apopka on (3H)17-beta-estradiol binding, these chemicals were mixed at concentrations measured in eggs in the competition binding assay. 2,2-bis(4-chlorophenyl)-N- (methoxymethyl)acetamide (p,p'-DDD) and trans-nonachlor, both found in Lake Apopka, interacted with aER, whereas others such as chlordane and toxaphene did not. Surprisingly, combinations of these chemicals decreased (3H)17-beta-estradiol binding in a greater than an additive manner. To assess the ability of chemicals to interact with aPR, competition binding assays with the synthetic progestin (3H)R5020 were conducted. Most of the chemicals tested did not reduce (3H)R5020 binding to aPR, whereas endosulfan, alachlor, and kepone inhibited binding. These results provide the first evidence that environmental chemicals bind the aER and aPR from the American alligator, supporting the hypothesis that the reported reproductive abnormalities may be related to the modulation of endocrine-related responses. The findings that combinations of chemicals demonstrated a greater than additive interaction with the aER and some chemicals bind to the aPR in the competition binding assay are novel. This suggests that interactions of these chemicals with the endocrine system are complex (Vonier P M; Crain D A; McLachlan J A; Guillette L J Jr; Arnold S F Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environmental Health Perspectives 104 (12). 1996. 1318-1322).

D. Evidence in Decrease of Urinary Ketosteroid

1. The dietary exposure to endosulfan at two different dose levels (12.5 and 37.5 mg/250g) daily to female goats for a period of 90 days caused a significant decrease in urinary 17-ketosteroid level in the treatment groups when compared to that of the control group (C = 3.27$\pm$ 0.22; T1 = 2.73 $\pm$ 0.14 and T2 = 2.49 $\pm$0.19), but was not significant between the two treatment groups. Decreased level was more perceptible on day 90 than day 45 when compared with day 0. The decrease in level may be associated with decreased hormone synthesis and hypofunction of the adrenal as well as ovarian glands. Thus, endosulfan might have adverse effect on the normal formation and metabolism of the sex hormones (Bose K K; Mukherjee S K; Prasad R L Indian J Anim Health, (1991). Vol. 30, No. 1, pp. 63-66).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

In-Vitro Testing

Estrogen Receptor Testing

Estrogens are hormones produced by humans and other animals; secreted primarily by the ovaries (female);

Estrogens are defined by their ability to induce the proliferation of cells of the female genital tract. Estrogens stimulate sexual maturation of females, and play an important role in reproductive function. Hormones act through specific receptors.

1. The E-SCREEN assay was developed to assess the estrogenicity of environmental chemicals using the proliferative effect of estrogens on their target cells as an end point. This quantitative assay compares the cell number achieved by similar inocula of MCF-7 cells in the absence of estrogens (negative control) and in the presence of 17 beta-estradiol (positive control) and a range of concentrations of chemicals suspected to be estrogenic. Concentration describes the dose at which an estrogenic effect is detected; maximal cell yield is obtained at concentrations between 10 and 100 pM estradiol. Most xenobiotics are active at 10M. The RPE (Relative Proliferative Effect) measures the ratio between the maximal cell yield achieved by the xenobiotic and that of estradiol. The RPP (Relative Proliferative Potency) is the ratio between the minimal concentration of estradiol and the minimal dose of the xenoestrogen test compound needed to produce maximal cell yields x 100. For endosulfan the concentration detected was at 0.0001 and maximum cell yield obtained at 10 M concentration. The Relative Proliferative Effect was between 77.17-81.25% that of estradiol (Soto, A.M, Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. , and Serrano, F.O.., 1995. Environ Health Perspect 1995 Oct; 103 Suppl 7:113-122).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

2. An "in culture" bioassay was used to assess the estrogenicity of several pesticides. The E-screen test uses human breast estrogen-sensitive MCF7 cells and compares the cell yield achieved after 6 days of culture in medium supplemented with 5% charcoal-dextran stripped human serum in the presence (positive control) or absence (negative control) of estradiol and with diverse concentrations of xenobiotics suspected of being estrogenic. Among the organochlorine pesticides tested, toxaphene, dieldrin, and endosulfan had estrogenic properties comparable to those of DDT and chlordecone; the latter are known to be estrogenic in rodent models. The E-screen test also revealed that estrogenic chemicals may act cumulatively; when mixed together they induce estrogenic responses at concentrations lower than those required when each compound is administered alone (Soto, A.M., Chung, K.L., and Sonnenschein, C., 1994. The pesticides endosulfan , toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ-Health-Perspect; VOL 102, ISS 4, 1994, P380-3).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

3. A yeast system highly and stably expressing a rainbow trout estrogen receptor (rtER) is used to analyze the biological activity of the receptor. The recombinant yeast system appears to be a reliable, rapid and sensitive bioassay for the screening and determination of the direct interaction between ER and estrogenic compounds. This system was used in parallel with a more elaborate biological system, trout hepatocyte aggregate cultures, to examine the estrogenic potency of a wide spectrum of chemicals commonly found in the environment. In hepatocyte cultures, the vitellogenin gene whose expression is principally dependent upon estradiol was used as a biomarker. Moreover, competitive binding assays were performed to determine direct interaction between rtER and xenobiotics. In this study, 50% of the 49 chemical compounds tested exhibited estrogenic activity in the two bioassays: the herbicide diclofop-methyl; the fungicides biphenyl, dodemorph, and triadimefon; the insecticides lindane, methyl parathion, chlordecone, dieldrin, and endosulfan; polychlorinated biphenyl mixtures; the plasticizers or detergents

alkylphenols and phthalates; and phytoestrogens. To investigate further biphenyl estrogenic activity, Its principal metabolites were also tested in both bioassays. Among these estrogenic compounds, 70% were able to activate rtER in yeast and hepatocytes with variable induction levels according to the system. Nevertheless, 30% of these estrogenic compounds exhibited estrogenic activity in only one of the bioassays, suggesting the implication of metabolites or different pathways in the activation of gene transcription. This paper shows that it is important to combine in vivo bioassays with in vitro approaches to elucidate the mechanism of xenoestrogen actions (Petit F; Le Goff P; Cravedi J-P; Valotaire Y; Pakdel F, 1977).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.

4. A number of chemicals released into the environment have the potential to interfere with physiological and developmental processes by disrupting endocrine pathways. Among the best known of these endocrine disruptors are compounds that mimic the action of the steroid hormone 17 beta -estradiol. These xenobiotic estrogens are believed to pose health risks to both humans and wildlife. Our laboratories are designing in vivo bioassays for xenobiotic estrogens based on induction of the egg-yolk precursor protein vitellogenin. Vitellogenin is normally produced by the liver of adult female non-mammalian vertebrates under estrogen stimulation. In immature or male animals, which have low levels of endogenous estrogens, vitellogenin can serve as a reliable biomarker for exposure to xenobiotic estrogens. Our model system used the African clawed frog, Xenopus laevis, an ideal species for laboratory screening of endocrine disruptors. Xenopus laevis vitellogenin was purified by diethylaminoethyl (DEAE) chromatography and used to generate polyclonal antibodies in rabbits. The resulting antiserum was used to develop an enzyme-linked immunosorbent assay (ELISA) for measurement of serum vitellogenin. Frogs were exposed to compounds by immersion in order to mimic environmental exposure to aquatic contaminants. Initially, frogs were immersed in the potent estrogenic agent diethylstilbestrol (DES) at a concentration of 1 ppm for 11 days to test the efficacy of the immersion protocol. Diethylstilbestrol exposed animals showed substantial induction of serum vitellogenin, indicating that the frogs are capable of responding to estrogenic agents present in their aquatic environment. Vitellogenin induction was then investigated for chlordane, dieldrin,endosulfan, and toxaphene, compounds that have been shown through in vitro assays to be weakly estrogenic when administered individually but more strongly estrogenic in combination. Adult male frogs were immersed in water containing the compounds (1 ppm, 11 days), both singly and in paired combinations. Endosulfan proved toxic at this concentration. Toxaphene- and dieldrin-treated frogs showed significant levels of vitellogenin induction, while chlordane-treated animals did not differ from controls. There was no evidence of a synergistic response between any of the combinations. This research demonstrates the utility of vitellogenin as a biomarker for exposure to estrogenic agents. The assays developed could be used to screen chemicals for estrogenic properties, to test waters for the presence of estrogenic agents, or to assess wildlife exposure to environmental estrogens (Palmer, B.D.; Huth, L.K.; Pieto, D.L.; Selcer, K.w. Environ. Toxicol. Chem., (19980100) vol. 17, no. 1, pp. 30-36.).

Note: An acceptable nonguideline study. The identity of the test compound and whether or not this study was conducted under GLP can not be verified.